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The molecular weight of ribonucleic acid prepared from ascites-tumor cells

Light-scattering measurements have been carried out on highly polymerized ribonucleic acid (RNA) prepared from ascites-tumor cells by the method of COLTER AND BROWN[1]. After extraction, the high molecular weight RNA was separated by precipitation with 1 *M* NaCl and kept at —40° until 12 h before the experiment. It was then thawed and dialyzed against pH 7.2 buffer (ionic strength (*I*), 0.02 sodium phosphate, 0.08 NaCl). The measurements were carried out in a 20 ml WITNAUER cell[2] with a BRICE photometer[3] with narrow-slit optics at angles between 25° and 145°. The solutions were clarified by centrifugation and filtration through a special filter[4]. Successive concentrations between 0.11 and 0.73 g/l were measured in a single cell using a micro burette[5] and mixing with a magnetic stirrer. Concentrations were measured by absorption at 260 mμ using the absorptivity of 21.0 lg^{-1} cm^{-1} obtained on an RNA sample dried to constant weight at 105°. The value of dn/dc (refractive index increment) was found to be 0.1716 and to be independent of concentration.

The results obtained gave a value of $10.05 \cdot 10^{-7}$ for $(Kc/R_{90})^{*}_{c=0}$ and an intrinsc dissymmetry of 1.14, resulting in a molecular weight of 1,090,000. (The experimental value for depolarization was found to be not greater than that obtained with bovine-serum albumin and, therefore, was not used in the calculations[6].) When the data were plotted according to ZIMM[7], the molecular weight was found to be 1,180,000 and the initial slope yielded a radius of gyration of 320 A. The curvature found in the zero-angle extrapolation plot suggests that the RNA is polydisperse. Analysis of the results in terms of the usual molecular models (random coil, sphere, rod and ellipsoid) showed that the data could be fitted reasonably only by rods or prolate ellipsoids. Using the light-scattering molecular weight of $1.1 \cdot 10^{6}$ and average particle lengths of 700–1,100 A, deduced from the intrinsic dissymetry and angular envelope of the scattering, along with a partial specific volume of 0.52[8], it is possible to calculate for prolate ellipsoids particle widths of 51–41 A This results in axial ratios of 14–27.

Electron micrographs, taken on similar preparations of RNA, have shown elongated particles which vary in both cross-section and length. The lengths of these particles ranged from 800 A to 2,000 A. They appeared to be tapered at the ends and the diameters measured at the center were between 40 and 70 A. These dimensions are considered to be in good agreement with those deduced from the light-scattering data.

A similar RNA preparation had an intrinsic viscosity of 0.40. Assuming 20% hydration and taking the light-scattering molecular weight of $1.1 \cdot 10^{6}$ and prolate ellipsoidal shape, one calculates from the data an axial ratio of 28 and dimensions of 40 A × 1,120 A. Such a hypothetical molecule would have a sedimentation constant of 29. Ultracentrifugal analysis at 0.03 g/l using ultraviolet optics presented a simpler picture than that obtained by schlieren optics[1]. Although there was some spreading of the boundaries, the RNA sedimented as two components, 60% with $s = 32$ and 40% with $s = 15$. The weighted average sedimentation coefficient ($s = 25$) of the

* K is an optical constant, c is the RNA concentration and R_{90} is the Rayleigh ratio at 90°.

RNA is in fair agreement with the value of 29 calculated from the parameters of the hypothetical prolate ellipsoid deduced from the light-scattering and electron-microscopy results and used to fit the data.

From these results it can be concluded that RNA of the ascites-tumor cell consists of compact globular molecules, best described as prolate ellipsoids of revolution. This would account for the low viscosity of its solutions and the high sedimentation coefficient observed. These results, however, have to be considered only as preliminary and subject to some uncertainty since RNA is unstable and subject to degradation both upon freezing and thawing and upon standing in solution at room temperature. Further work is in progress with the aim of eliminating these complications.

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The nature and action of mitochrome

POLIS AND SHMUKLER[1] recently described the preparation from disintegrated mitochondria of a purified protein which activated the latent adenosine triphosphatase (ATP-ase) and uncoupled oxidative phosphorylation in freshly prepared mitochondria. Since the absorption spectrum of this preparation resembled that of a haem compound, the authors gave it the name mitochrome. These findings are of considerable interest, since it seems probable that mitochrome is responsible for the development of ATP-ase and loss of phosphorylative activity during the aging of mitochondria.

To study the relationship between this haem compound and the uncoupling of oxidative phosphorylation by the mitochrome preparation, the latter was prepared[1] and tested as an inhibitor of the ATP-inorganic phosphate exchange reaction in the presence of various substances known to react with haem compounds in general and mitochrome in particular. Table I shows that mitochrome inhibited the exchange almost completely, while the other substances were without effect, either in the presence or in the absence of mitochrome. The results indicate that under our conditions the exchange reaction is independent of the state of oxidation or reduction of components of the respiratory chain, suggesting that a haem is not directly involved in the exchange reaction. Since $Na_2S_2O_4$, $K_3Fe(CN)_6$, CO and KCN had no effect on the inhibition of the exchange reaction by the mitochrome preparation, whereas these compounds caused marked changes in the absorption spectrum[1], it became doubtful whether the haem could be responsible for the inhibitory effect. It was also found that the inhibitory activity was not lost if a fine suspension of heat-treated (10 min at 100°) mitochrome was used. It may be concluded therefore that neither protein nor haem but another component of the preparation must be the active inhibitor.

It was found that this substance was readily extracted by isooctane, which caused little precipitation of protein or alteration in the pigment as evidenced by the unchanged spectrum of mitochrome after extraction. The isooctane was evaporated in the absence of air and the residue dissolved in 96% ethanol. It can be seen from Expt. 2 that the isooctane extract caused a marked inhibition of the exchange reaction and that the extracted mitochrome had no effect. In other experiments, it was found that this inhibition could be abolished by albumin and that the isooctane extract also strongly inhibited the phosphorylation coupled to the oxidation of succinate.